R E M A R K S

The amendment to claim 1 is supported in the specification on page 25, lines 22 to 23.

With respect of Rule 116, entry of the above amendment to claim 1 is respectfully requested, since the amendment is in reply to the Examiner's statement on page 4, lines 6 to 10 of the final rejection.

The presently claimed invention concerns a method of analyzing a target nucleic acid by applying a nucleic acid amplification reaction to a test solution, wherein an amplified product is labeled with a marker molecule. The method comprises:

(a) performing a nucleic acid amplification reaction of the target nucleic acid in a test solution containing a forward primer and a reverse primer, a substrate comprising nucleotides, a nucleic acid polymerase and a target nucleic acid, wherein the number of one of the forward primer and the reverse primer is lower than that of the other primer, and the primer present in a lower number is labeled with a marker molecule capable of generating a detectable signal to form a labeled primer, the nucleic acid amplification reaction being performed until the primer present in a lower number is consumed;

(b) measuring a signal from the marker molecule in the test solution after initiation of the nucleic acid amplification reaction;

(c) evaluating a fluctuation motion of the amplified nucleic acid which is labeled with the marker molecule, in the test solution on the basis of the signal detected; and

(d) quantifying the target nucleic acid on the basis of evaluation results.

In the presently claimed invention, the evaluation of a fluctuation motion of a labeled nucleotide by fluorescence correlation spectroscopy ("FCS") is skillfully combined with an asymmetrical polymeric chain reaction ("PCR"), and this combination permits quantitative measurement of the initial amount of a target nucleic acid contained in a sample, which was not possible heretofore. To achieve this advantage, in the presently claimed invention, the concentration ratio of the claimed two primers and the labeling criterion are important, as described in the present specification.

According to applicant's claimed method, an amplified double stranded nucleic acid product is labeled with a marker molecule without fail, whereas a single-stranded nucleic acid product is not labeled (see Fig. 3 of the present application, which is

reproduced hereinbelow). The reaction can be observed by fluorescence correlation spectroscopy. In this manner, the presently claimed invention makes it possible to analyze a target nucleic acid.

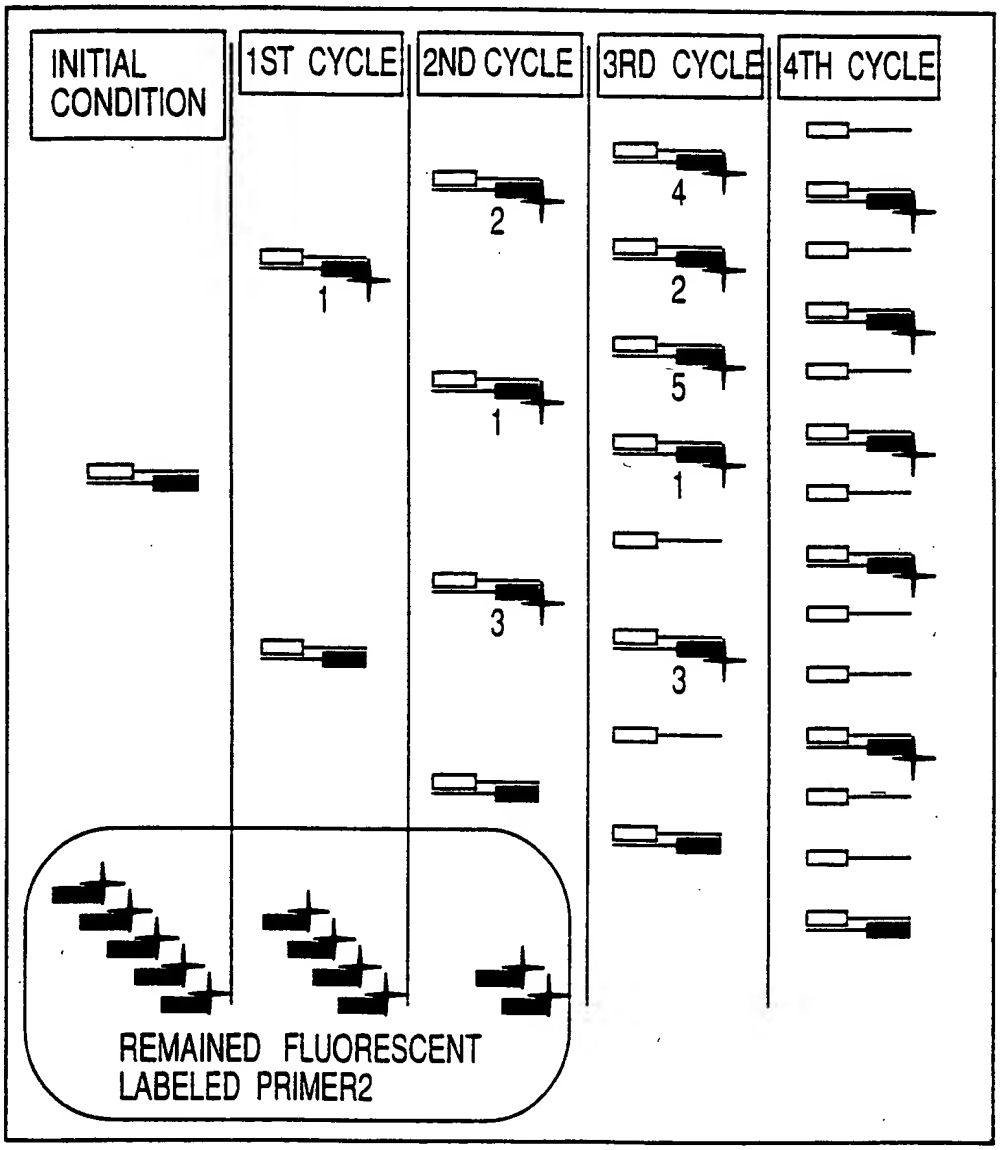


FIG. 3

Claims 1 to 5, 7, 8, 39 and 42 to 43 were rejected under 35 USC 103 as being unpatentable over Salituro et al. USP 6,391,544 in view of Eigen et al. USP 5,807,677 for the reasons set forth in Paragraph No. 1 on pages 2 to 4 of the Office Action.

It was admitted in the Office Action that Salituro et al. do not disclose any of (i) evaluating a fluctuation motion of the amplified nucleic acid, (ii) quantifying the target nucleic acid and (iii) the specific ratio of the concentration of the primers, as recited in applicant's claims 42 and 43.

Salituro et al. do not disclose the same asymmetric PCR as in the presently claimed invention. Claim 1 of Salituro et al. recites the following:

> "wherein the improvement comprises providing the first primer sequence in 15% to 250% excess over the second primer and wherein a probe is hybridized to the amplification product from the first primer to form a hybrid complex, and the hybrid complex is detected as an indication of the presence of the target sequence in the test sample" (emphasis supplied).

More specifically, the asymmetric PCR of Salituro et al. is carried out similarly to the asymmetric PCR of Gyllensten et al. (cited in a previous Office Action). In this method, a double-

stranded DNA and a single-stranded DNA are produced by PCR amplification. Only the single-stranded DNA is then detected by a probe.

Furthermore, Salituro et al. describe the following in column 10, lines 5 to 10:

> "Only when the unlabeled primer was present at concentrations lower than the labeled primer was a more linear signal produced correlating with target concentration."

As is apparent from above, in Salituro et al., the first primer present in a larger amount is labeled. In contrast thereto, in the presently claimed invention, a primer present in a lower amount is labeled. Hence, the asymmetric PCR of Salituro et al. is completely opposite to that of the presently claimed invention.

In the method of the presently claimed invention, only when the amount of labeled primer is lower than that of unlabeled primer, the phenomenon where free-labeled primer is consumed with the progress of a PCR amplification reaction is observed. Thus, even if the primer which will never be consumed is labeled, significant data for the Salituro et al. method cannot be obtained.

Applicants respectfully disagree with the position taken on page 4, lines 4 and 5 of the Office Action for the following reasons.

Column 8, lines 18 to 21 of Salituro et al. state that "the unlabeled primer is present at concentrations below that of the labeled primer." As noted above, column 10, lines 5 to 6 of Salituro et al. state that "the unlabeled primer was present at concentrations lower than the labeled primer." Thus, column 8, lines 8 to 21 and column 10, lines 5 to 6 of Salituro et al. clearly disclose the relationship of "unlabeled primer < labeled primer" concerning the concentration ratio of primers. This relationship disclosed in Salituro et al. is opposite to the relationship of "unlabeled primer > labeled primer" recited in the claims of the present application.

In the presently claimed invention, the amount of target nucleic acid contained in a sample before the PCR amplification can be determined based on the number of PCR cycles carried out, until the labeled primer is completely consumed (see FIG. 3 (which is reproduced hereinabove) and page 25, lines 22 to 23 and page 29, lines 1 to 5 of the present specification).

In summary, in contrast to the presently claimed invention, Salituro et al. disclose an asymmetric PCR where a primer present

in a larger amount is labeled.

Eigen et al., in column 3, lines 46 to 57, discuss the advantages of fluorescence correlation spectroscopy ("FCS") over the polymerase chain reaction ("PCR"). However, as admitted in the Office Action, Eigen et al. do not teach or suggest combining FCS with PCR.

It is respectfully submitted that one of ordinary skill in the art would not consider to combine Eigen et al. and Salituro et al., since Eigen et al. do not teach to combine FCS and PCR.

Each of Salituro et al. and Eigen et al. do not disclose a method for the quantitative measurement of the initial amount of a target amino acid. Therefore, even assuming *arguendo* that these two references are combinable, it would not be possible to arrive at the present invention, in which a quantitative measurement of the initial amount of a target nucleic acid contained in a sample is made by skillfully combining the measurement of a fluctuation motion of a labeled nucleotide by FCS with asymmetrical PCR. Moreover, if one of ordinary skill in the art considered to combine the FCS of Eigen et al. with the PCR of Salituro et al., the presently claimed invention would not be reached because the PCR of Salituro et al. is substantially

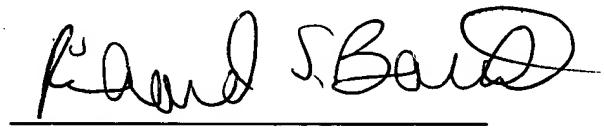
different from the PCR in the presently claimed invention.

It is therefore respectfully admitted that applicant's claimed invention is not rendered obvious over the references, either singly or combined in the manner relied upon in the Office Action, in view of the distinctions discussed hereinabove. It is further submitted that there are no teachings in the references to combine them in the manner relied upon in the Office Action.

Reconsideration is requested. Allowance is solicited.

If the Examiner has any comments, questions, objections or recommendations, the Examiner is invited to telephone the undersigned at the telephone number given below for prompt action.

Respectfully submitted,



RICHARD S. BARTH
REG. NO. 28,180

FRISHAUF, HOLTZ, GOODMAN & CHICK, P.C.
767 THIRD AVENUE - 25TH FLOOR
NEW YORK, NEW YORK 10017-2023
Tel. Nos. (212) 319-4900
(212) 319-4551/Ext. 219
Fax No. (212) 319-5101
E-Mail Address: BARTH@FHGC-LAW.COM
RSB/ddf